

The nucleosome repeat length of *Kluyveromyces lactis* is 16 bp longer than that of *Saccharomyces cerevisiae*

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Nucleosomes form the first stage in the condensation of eukaryotic DNA into chromosomes. The nucleosome core particle consists of 146 bp of DNA wound around a histone octamer, consisting of pairs of histones H2A, H2B, H3 and H4. Subsequent nucleosome core particles are joined by linker DNA, resembling 'beads on a string' under the electron microscope. The length of the linker DNA varies between approximately 10 bp for lower eukaryotes, like *Aspergillus nidulans*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, and up to 100 bp in sperm of several invertebrates (1, 2, 3, 4, 5). Binding of histone H1 to the linker DNA plus nucleosome results in a particle called chromatosome. In a chromatosome 166–168 bp of DNA are protected against limited digestion by micrococcal nuclease, which cleaves preferentially between nucleosomes (6).

S.cerevisiae and *S.pombe* are reported to have nucleosome repeat lengths of approximately 160 and 156 bp, respectively, which are too short to accommodate histone H1 (2, 3, 4, 5) and so far, a histone H1 homologue has not been identified in these species. We are interested in the process of chromosome segregation and have chosen *Kluyveromyces lactis* as a model system. *K.lactis* is a budding yeast like *S.cerevisiae* and is a suitable organism for the production of heterologous proteins like prochymosin (7), human serum albumin (8), and human interleukin 1 β (9). It has six chromosomes versus sixteen for *S.cerevisiae* and three for *S.pombe*. *K.lactis* centromeres resemble those of *S.cerevisiae* on the sequence level, but both centromeres cannot be functionally exchanged (10, 11). *S.pombe* centromeres are completely different (12), reflecting the phylogenetical distance between the fission yeast *S.pombe* and the more closely related budding yeasts *K.lactis* and *S.cerevisiae* (13).

During the investigation of the chromatin structure of *K.lactis* centromeres, we observed an interesting difference in the nucleosome repeat lengths of *K.lactis* and *S.cerevisiae*, which were determined from micrococcal nuclease digestions of nuclei. The experimental procedures given below, are based on a method described by Bloom and Carbon (14).

K.lactis strain JBD100 (*trp1 lac4-1 ura3-100* (10)) and *S.cerevisiae* strain 15C (*MAT α leu2-31,12 ura3-52 his4-580 Δ trp1 pep4-3* (15)) were grown in 1 liter of YPD medium (16) to an optical density of 1.5. For practical purposes the cells were divided over four tubes. They were washed twice with sterile water, once with TESM (0.2 M Tris, 0.1 M EDTA, 1.2 M Sorbitol, pH = 9.1), after which each pellet was resuspended in 125 ml TESM. After 10 minutes at room temperature, the cells were spun down, each pellet washed with 225 ml SCE (1

M sorbitol, 0.1 M citric acid, 0.06 M EDTA, pH = 5.8), and resuspended in 25 ml SCE. Here the four tubes were combined and 1 ml of glusulase (NEN (Dupont) Research Products) or 20 mg Zymolyase 100T (Seikagaku Kogyo Co. Tokyo) were added to spheroplast the cells. Subsequently, the spheroplasts were pelleted (in 4 tubes again), each pellet washed with 200 ml SPC (1 M Sorbitol, 20 mM Pipes, 0.1 mM CaCl₂, pH = 6.3), and resuspended in the remaining liquid. The suspensions were each added to 25 ml of Ficoll–PMSF solution (9% Ficoll 4000, 20 mM Pipes, 0.5 mM CaCl₂, pH = 6.3 supplemented with 25 ml/l of a PMSF-solution containing 6.9 mg/ml 95% ethanol). The lysed spheroplasts (= nuclei) were pelleted, washed with a SPC–PMSF solution (25 ml PMSF solution added to 1 liter of SPC), and resuspended each in 1 ml SPC–PMSF. The nuclei were preincubated at 32°C for 3 minutes, after which a control (non-digested) sample was taken. Micrococcal nuclease (Pharmacia) was added (200–500 U/ml nuclei suspension), and the digestion allowed to proceed at 32°C. Samples were taken after 0, 0.5, 1.5, 5, and 15 minutes. Reactions were terminated with half a volume of SEN (2.5% SDS, 125 mM EDTA (pH

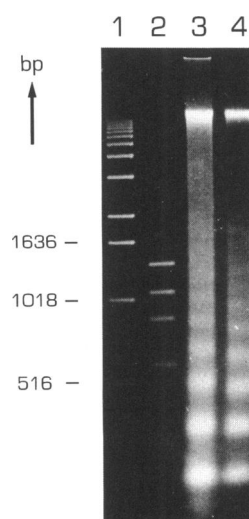


Figure 1. Nucleosome ladders of *K.lactis* and *S.cerevisiae*. *K.lactis* and *S.cerevisiae* chromatin was digested with micrococcal nuclease for 15' (278 and 250 U/ml nuclei suspension, respectively) and run in a 1.4% agarose gel. Lane 1: 1 kb marker; lane 2: Φ X174 cleaved with *Hae*III; lane 3: *K.lactis*; lane 4: *S.cerevisiae*.

= 8), 2.5 M NaCl). The nuclei were left at -20°C for sixteen hours, after which they were extracted several times with phenol/chloroform and ethanol precipitated. Subsequently, they were treated with RNase A, followed by Proteinase K (both from Boehringer Mannheim GmbH), extracted again with phenol/chloroform and finally ethanol precipitated. Portions of the chromatin samples were analyzed on 1.4–2.0% agarose gels containing either $1\times\text{TBE}$ or $1\times\text{TAE}$ (17).

Figure 1 shows the micrococcal nuclease digestion patterns of *K.lactis* and *S.cerevisiae*. The nucleosome repeat lengths were estimated in two ways: (A) the size in bp of a polynucleosome (5-, 6- or 7-mer) was divided by the number of nucleosomes, giving the number of basepairs per nucleosome plus linker DNA; (B) the average of the distances between the respective bands was taken as the nucleosome repeat length (2).

Using method (A), the *K.lactis* nucleosome repeat length varied between 170 and 176 bp, depending on the period of digestion. Thomas and Furber (2) have reported a similar effect on the *S.cerevisiae* nucleosome repeat length. The advantage of method (B) is that the distance between sequential nucleosomal bands is independent of the extent of digestion as long as the nucleosomal core remains intact. With method (B) a value of $171 \pm$ was found ($n = 21$), obtained from several samples digested with different amounts of micrococcal nuclease. For *S.cerevisiae*, the nucleosome repeat length was determined at 157–160 using method (A) and 155 ± 5 ($n = 25$) using method (B). We favor method (B) since it is less dependent on the degree of digestion, unlike method (A), which gives smaller values when the DNA is more heavily digested. The obtained values correspond reasonably well to the 160–165 bp reported previously (1, 2, 3). Recently, Bernardi *et al.* (4) found values of 162 and 156 bp for tri- and tetranucleosomes, respectively.

The nucleosome repeat lengths of *K.lactis* and *S.cerevisiae* were always determined from adjacent lanes, using the same method for both yeasts. The difference of 16 bp is genuine, since the same values for the nucleosome repeat length were found routinely in different gels, with varying amounts of DNA loaded, from several micrococcal nuclease digestion time points.

The observed difference of approximately 16 bp in nucleosome repeat length between *K.lactis* and *S.cerevisiae* is surprising in view of the close phylogenetical relationship between the two species (13). Whereas the linkers of *S.cerevisiae* and *S.pombe* are too short to allow the formation of chromatosomes, the linker of *K.lactis* is long enough, leaving the interesting possibility that *K.lactis* has a histone H1 homologue or a H1-like protein.

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